

**TAXONOMIC STATUS OF THE SONOYTA MUD TURTLE
(*KINOSTERNON SONORIENSE LONGIFEMORALE* IVERSON)
BASED ON MITOCHONDRIAL D-LOOP SEQUENCE,
WITH A DISCUSSION OF PHYLOGEOGRAPHY**

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Abstract

The Sonoya Mud Turtle (*Kinosternon sonoriense longifemorale* Iverson) occurs as an isolated population in the most arid part of the species' range. Evidence of small population size and possible decline, isolation, known habitat degradation, and a lack of key information, have led to consideration of listing this taxon as threatened or endangered, or at least preparing a conservation plan for it. Before either step occurs, we need further or confirmatory information on occurrence, abundance, population trends, and validity of the taxon. In this report I provide information on the populations now known for this taxon and evaluate its validity by studying a 961 base-pair portion of the d-loop on the mitochondrial genome. There are presently 8 known localities, with a rough preliminary estimate of 1,200 individuals: it seems unlikely there could be less than 600 or more than 2,700.

Analysis of the d-loop confirmed Iverson's separation of *K. s. longifemorale* from *K. s. sonoriense*, although it was not possible to collect specimens from the portion of the latter's range in Sonora. Little variation was evident in the gene sequences in our samples, but the few informative sites suggested that the Sonoya Mud Turtle, although fully diagnosable based on our data, might be paraphyletic with respect to the nominate subspecies. An alternative hypothesis is presented suggesting brief re-contact between the differentiating subspecies at some earlier time.

Introduction

Iverson (1981) described the mud turtles from an isolated desert region of southwestern Arizona and northwestern Sonora as a new subspecies, the Sonoya Mud Turtle, *Kinosternon sonoriense longifemorale*, leaving all other populations in the nominate subspecies of the Sonoran Mud Turtle *K. s. sonoriense* (LeConte). [Note that the common name of this species has been altered to Sonora Mud Turtle, incorrectly implying that its occurrence is Sonora, Mexico; I recommend the earlier usage, Sonoran Mud Turtle (Lowe, 1964; Stebbins, 1985), which correctly implies that this is a species of the Sonoran Desert region and Sonoran life zones]. Iverson's (1981) description was derived from multivariate analysis of morphometric ratios from turtles throughout the range of the *Kinosternon hirtipes* Species Group (*K. hirtipes* and *K. sonoriense*) based on a set of 19 shell measurements. The Sonoya Mud Turtle appeared to be rather strongly distinctive using step-wise discriminant functions methods. It was diagnosed based on the long femoral scute and concomitantly short anal scute on the plastron, as well as the wide first vertebral (carapace) and narrow gular (plastron) scutes.

Iverson's (1981) material was from Quitobaquito, in Organ Pipe Cactus National Monument (ORPI), Pima County, Arizona, and from Rio Sonoya, Sonora, south of ORPI, and included the holotype (USNM 21710) collected by Edgar Mearns in 1894 from an artificial

pond fed by springs at Sonoyta, Sonora. Paratypes included 3 others from 1894 collected from Rio Sonoyta 3 mi from Sonoyta. These 1894 localities may no longer exist as turtle population sites. Rio Sonoyta has been subjected to groundwater pumping and erosion, yielding greatly lowered water tables and an incised channel that now only flows as a perennial stream at the site of Agua Dulce, now called Papalote. Turtles at Quitobaquito have been protected within ORPI, but management efforts at Quitobaquito ignored the turtles until the 1980's, apparently leading to a population decline (Rosen, 1986; Rosen and Lowe, 1996). The population at Quitobaquito was considered small (around 100 individuals), and little was known of the status of this taxon in Sonora, except that it was seen at Sonoyta and Papalote during the 1980's.

As a result of concerns about the status of this taxon, the Sonoyta Mud Turtle Conservation Team was convened by Jim Rorabaugh of U.S. Fish & Wildlife Service (USFWS) on June 19, 1997, at ORPI. Funding was obtained and re-censussing carried out at Quitobaquito in 1999 (Rosen, 2000). It became apparent that successful long-term conservation would only be possible if populations were identified and preserved in Mexico, as well as at Quitobaquito. As a result of the evidently small population sizes and other potential problems facing all populations, USFWS identified the Sonoyta Mud Turtle as a Candidate for listing as threatened under the Endangered Species Act of the United States (see Knowles et al., 2002a & b).

Sampling and initial mark-recapture efforts during 2001-2 confirmed small but significant populations at 7 localities at 4 general sites in Sonora (Paredes and Rosen, 2003 *in review*), and confirmed a relatively small but stable population remaining at Quitobaquito (D. Riedle, 2003 *presentation*). This confirmed potential problems that could lead to listing of the taxon as a threatened or endangered species, or to a Candidate Conservation Plan in lieu of listing. Insofar as Rosen (1987, *and unpublished*) believed there to be significant differences in shell and egg shape among populations of *K. s. sonoriense*, the potential for listing led to a perceived need to re-examine the taxonomic status of the described subspecies to ensure that a valid entity was being considered. The purpose of the present report is to describe genetic data results, which demonstrate the distinctiveness of the Sonoyta Mud Turtle and validate Iverson's (1981) description of *K. s. longifemorale*.

Methods

Sonoyta Mud Turtle Population Areas

Turtles have been found at Quitobaquito plus 7 sites in Mexico (Fig. 1). The following is a brief description of each site and its population.

1. Quitobaquito. This site is well-known and described. Current results (Riedle, 2003) indicate a population estimated at 124, lower than the mid-1990's peak, but not indicating a major decline. Riedle also reported recruitment of females into the adult population, possibly alleviating earlier concerns about the sex ratio of adults (Rosen and Lowe, 1996).

2. Rio Sonoyta at Presa Xochimilco. This is a sizeable dam pool approximately 1.5 km east of Mexican Highway 2 bridge over Rio Sonoyta. The dam is a large (ca. 6 m tall) stone and concrete structure established during the middle of the 20th century. It backs up a runoff-filled pool about 5-9 m across and up to 1 km long, or longer. The dam is mostly filled with sediment, and the maximum water depth when full is from 1-2 m. During 2001-2002, this pond filled readily and dried quickly (from full in September 2002, following runoff after severe drought, to almost entirely dry a month later).

The environment around the pond has an open stand of willow and cottonwood, and an

open mesquite-saltcedar bosque. The deepest area, near the dam, is planned for development as a park for Sonoyta, and during early 2002 thickets of saltcedar and mesquite bosque were chopped out and bladed on the north side of the pond. The open bosque to the east, and the saltcedar-mesquite-willow-seepwillow stands on the south side were not disturbed. A herd of ca. 40 goats passes through and browses this area at least once per day.

About 0.5 km upstream from the dam, a major army barracks discharges wastewater onto the south side gallery floor and into the pond bed, creating large perennial pools that apparently sustain the turtles, and perhaps black bullhead catfish, even during periods of drought. In 2001, mosquitofish were abundant in this region, but in 2002 none were seen. Gray foxes were abundant.

Turtles are reasonably abundant and prominent throughout this area when water is present. Trapping has been carried out along about 0.7 km of this pond, although systematic resampling was not done during 2002, primarily due to drought and poor water quality. Recaptures obtained have suggested population size at about 270 turtles. It is possible there are turtles further upstream, beyond the effective reach of our sampling, and that this estimate is somewhat low. No sign of recruitment after the mid-1990's El Niño was detected.

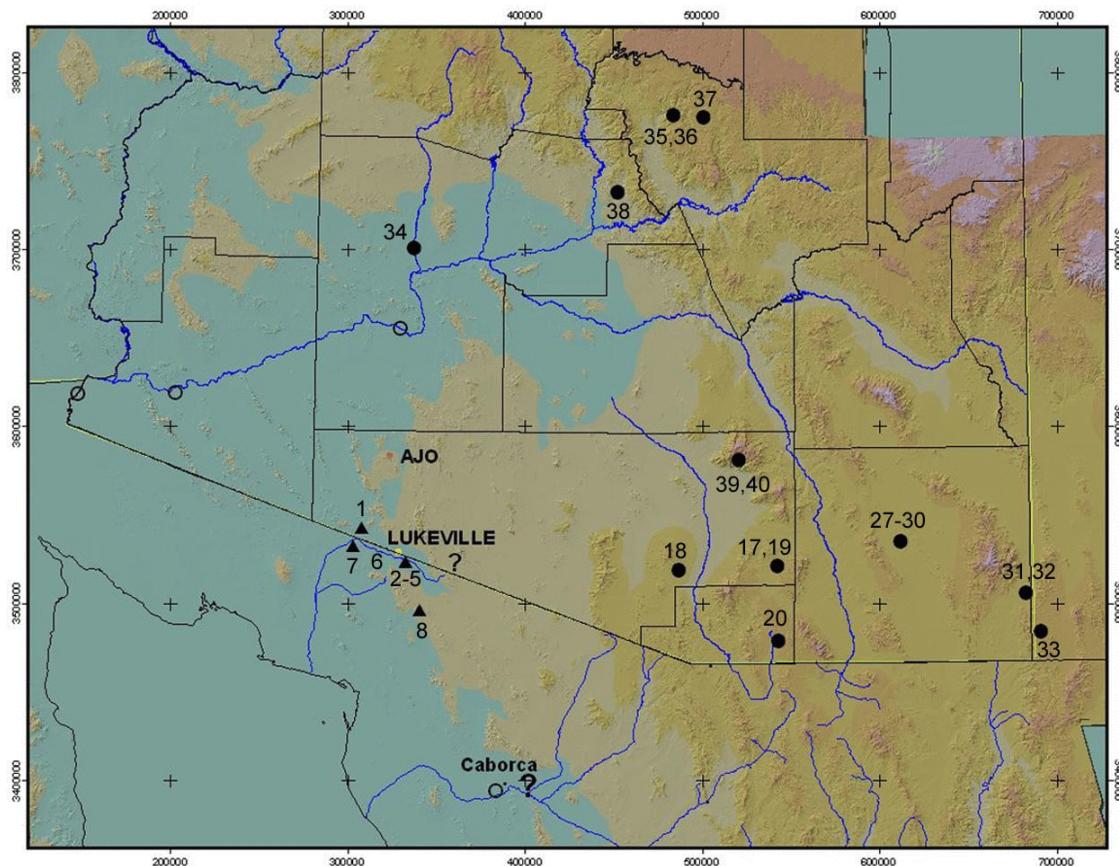


Fig. 1. Major population sites for the Sonoyta Mud Turtle (*Kinosternon sonoriense longifemorale*, triangles; numbers correspond to Methods text), sources of genetic samples for the nominate subspecies (*K. s. sonoriense*, solid circles; numbers correspond to Table 1) and apparently extirpated populations of the Sonoran Mud Turtle (*K. sonoriense*, hollow circles). Large channels of major drainages are shown in blue.

3. Rio Sonoya below Presa Xochimilco. Below the dam the stream bed lies on exposed bedrock of the Sonoya Hills and appears to include a small series of about 5 or 6 naturally perennial spring-fed pools. These are small pools at low water, estimated at 5-12 m X 2-4 m and generally 1 m deep or less. They are concentrated within about 0.5 km of the dam, and there is raw wastewater, from private houses above the deeply incised bed, discharging directly to one or two areas, creating and supplementing perennial water. The surroundings are a very dense saltcedar thicket with some mesquite, especially peripherally, and occasional willows and cottonwoods.

During times of higher water, there may be lasting flow connecting all these pools and extending well below them. During our first trip (October 2001) there was also water at the Highway 2 bridge and at the road crossing about 0.5 km below that, in Sonoya. However, in fall 2002, the only water was in the few perennial pools and at a very small discharge point at the east abutment of the highway bridge.

Turtles inhabit this entire reach. It is not clear whether they move down to the lowest road crossing area from the perennial reach, but in fall 2002 none were seen at the highway bridge area. The total population is presumably not large, but no quantitative estimate is available for it. During October 2002, our traps at the spring pools were raided within one hour of being set, and it appears that turtles may have been taken for sale somewhere in Sonoya. Trapping in this area will require labor-intensive oversight of the equipment. Its objective would be to determine whether this segment is somewhat independent of the Presa Xochimilco population. A guess at the population of this entire area is 75 turtles.

4. Rio Sonoya above Presa Xochimilco. Shallow water in pools was found in October 2001 about 3 km upstream of Xochimilco, at the north base of the Cemetery Hills east of Sonoya. These were small pools on a sand-silt-clay substrate set in dense to open riparian thicket that was used as horse pasture. Also during this trip, even smaller pools were seen on Rio Sonoya north of Ejido Desierto de Sonora, and pools with mosquito fish were found in the riverbed at the large Ejido near La Nariz, where local residents reported that turtles were seen in the river. However, turtles were confirmed only at Cemetery Hill pasture, which was trapped. Although no estimate for the number of Sonoran Mud Turtles present in these reaches can be made, it would appear the number would be quite small.

5. Sonoya Sewage Lagoon. This is a large pond (> 5 ha) on the west side of Sonoya near Rio Sonoya. It was located inadvertently by following the source of emerald green water found in the riverbed. The main lagoon is a large expanse of open water with a fringe of mesquite, broom, and various other Sonoran Desert shrubs. Numerous turtles were observed surfacing and basking during a brief visit in October 2001. There is a second, adjacent lobe of the sewage lagoon system, where wastewater enters the system. The air is quite foul in this area, the environment is densely choked with cattails, and turtles were not seen during a very brief examination. Based on the number of turtles seen and the extent of habitat, perhaps 300 turtles may inhabit the Sewage Lagoon.

6. Rio Sonoya at Ejido Santo Domingo and Josefa Ortiz. This site is several kilometers upstream of the Papalote reach, and can be located by finding the choke point within hills (Lomas Santo Domingo) mostly south of Rio Sonoya and a small set of low volcanic hills north of the river. In October 2001 a single soup-bowl sized water remnant was located at the point of the low hills, below a point where a saltgrass flat outside the river gully walls demonstrates shallow groundwater. One Sonoya Mud Turtle was seen on the sand next to this spot of water, and an ejido member said that more water may be seen when the saltcedar loses its leaves, and then other turtles may be found in the stream. An attempt to confirm this was made in March 2002, and indeed found more water; however, no turtles were captured. There are probably only a

handful of turtles living in this part of the river, and that probably constitutes all of the turtles between Sonoyta and Papalote.

7. Papalote (Agua Dulce) Reach of Rio Sonoyta. This site is a perennial stream segment about 1.5 - 3 km long, within a shallowly incised floodplain as it passes through the Quitobaquito Hills south of Quitobaquito, having been deflected northward by the bajada of Sierra Los Tanques/Cipriano. The stream is dominated by runs and gentle riffles, which generally vary from 1-2 m wide and 15-30 cm deep. The flow is punctuated by regular but widely spaced corner pools of 1-1.5 m X 2-3 m and 0.4-0.9 m in the deepest spot. These pools tend to have good cover in deeply undercut banks stabilized by seepwillow and saltcedar roots. Other pools are little more than deep runs, with weakly undercut banks stabilized by Bermuda grass and spikerush. The surrounding environment is dense saltcedar thicket, fringed by mesquite, saltbush and alkali scrub, and smoketree.

The area is moderately grazed, with cattle preventing the stream margins from stabilizing and producing deeper pools, which is probably good for the native fishes but less than optimal for turtles. Longfin dace (*Agosia chrysogaster*) are abundant in the main flow, Quitobaquito pupfish (*Cyprinodon (macularius) eremus*) are abundant on the flow margins, mosquitofish (*Gambusia affinis*) are abundant in shallow, still margins, and black bullheads (*Ameiurus melas*) are uncommon in the pools. I saw this same fish assemblage in this reach in 1983 and 2001-2. The abundance of dace and pupfish is truly impressive. The drought of summer 2002 took a toll on the fishes, especially the dace, but all species persisted in numbers.

Turtles are moderately uncommon in this reach, and difficult to observe. They appear to use undercut banks with burrows that go far back into the embankments, and into which they may rapidly disappear. All ages of turtles were observed, and a highly preliminary estimate of 108 individuals was made. This is probably an underestimate, since the full length of stream was not effectively trapped, and visual observation and hand capture were remarkably ineffective, both night and day. There are probably 200 turtles in this reach.

8. Quitovac. This is a set of 4 adjacent springs feeding a moderately large (1+ ha) lagoon situated in the middle of paloverde-mixed columnar cactus desert near the head of a portion of the Rio Sonoyta basin that is highly isolated and to the southeast of the mainstem. This sub-drainage trends south, away from the mainstem, and approaches it only in the sands near the Gulf of California. It is the site of a Tohono O'odham village with several tidy streets, a new school, and a small, venerable orchard of pomegranate and fig trees (Nabhan et al., 1982).

The hard, calcareous water emerges from gently sloping terrain into springhead pools, and flows down narrow channels to the lagoon, which is contained by a mesquite-clothed levee anchored to a mass of limestone. The environs are of open mesquite and alkali scrub, as well as palms, scattered willow, a small boggy spot, and the orchard and grassy outflow plain. The area is fairly heavily used by livestock, especially horses. The water is full of mosquitofish and redbelly tilapia (*Tilapia (Oreochromis) zillii*).

Substantial numbers of turtles were seen in visits made in March and October of 2002. Turtles were marked only on the last trip, but as a first guess it may be expected that 200 turtles inhabit the site.

9. Total Number of Sonoyta Mud Turtles (Preliminary Estimate). At Sonoyta, a reasonable estimate suggests that there exist somewhere in the neighborhood of 700 turtles, whereas at Papalote and Santo Domingo a total of 200 may occur. With another 200 at Quitovac and 124 at Quitobaquito, it would appear the total population of this taxon may be in the neighborhood of

1200 individuals. This must be considered a rough estimate: it is highly unlikely that there are less than 600 or more than 2700 turtles.

The subpopulations cannot be considered secure, although none are known to be in immediate danger. In particular, the bulk of the population at Sonoya may continue to thrive or, if wastewater management changes without regard to the turtles, the population could be reduced to nil in short order, as is apparent at Tucson and Caborca, the latter site having been visited in October 2002. Groundwater pumping could draw down the Agua Dulce reach and eliminate that population. Unforeseen developments (agriculture, mining) could eliminate the population at Quitovac. Thus, there is a plausible scenario in which Quitobaquito could be the only population site remaining as it is today.

Genetic Sample Collection

Genetic samples were collected by withdrawing 0.2-0.4 cc of blood from the sub-nuchal/first vertebral sinus using disposable 1-3 cc syringes with small gage (0.4-0.5 X 13-25 mm; 25G1 - 27G ½) needles. Samples were injected into 500 L Microtainer!" brand (purple top) tubes coated with EDTA, which were labeled with permanent marker and stored individually in labeled Ziploc!" bags and protected from heat. Some additional samples consisted of toe clips and blood stored in full-strength ethanol. For the Sonoya Mud Turtle samples, turtles were marked prior to release, whereas other turtles, from non-study populations, were released unmarked. The origin of samples that were successfully sequenced (Table 1, Fig. 1) included 3 sites within the expected range of *K. s. longifemorale* and 10 sites spanning much of the distribution of *K. s. sonoriense* in Arizona and New Mexico (Table 1). With Rafaela Paredes and Israel Barba, I attempted to locate a source for samples in Caborca, in the Concepcion basin, but found no population locality.

Laboratory Methods

Tissue samples from the Sonoran Mud Turtle samples (Table 1) were extracted using a standard phenol-chloroform extraction. The control region of the mitochondrial genome was amplified by the polymerase chain reaction (PCR) with ChelProf (Allard et al., 1994) and 12S-57R primers (Serb et al., 2001). Fragments were sequenced on an ABI PRISM 3700 DNA Analyzer (Applied Biosystems) using KNCR 271F (Serb et al. 2001), DLS (5'GTGAAGAGCAGGACATC3') for the area before the AT-region and DLE (5'GGTTAGGACTAAGTCTT3') primer for the area after the AT-region.

The sequences derived from this procedure (Appendix) were aligned with sequences for a set of outgroups (*Kinosternon flavescens*, *K. subrubrum*, and *Stenotherus odoratus*) available in the published literature (Serb et al. 2001) using Sequence Navigator 1.0.1 (PE Applied Biosystems) and then compiled in McClade 4.03 (Maddison and Maddison 2001). After excluding the AT-region, sequences were 961 base pairs long. Phylogeny of this group of sample sequences was inferred using parsimony in PAUP 4.0b10 PPC (Swofford 2001).

A heuristic search of 1,000 random-addition replicates was conducted using TBR branch-swapping in PAUP (Swofford 2002). Only 6 sites were variable within the *K. sonoriense* samples, so bootstrap analyses were not conducted. One most-parsimonious tree was found (treelength = 148).

Table 1. Origin and parameters for blood and tissue samples used in genetic analysis of the Sonoran Mud Turtle.

Sample Number	Putative Taxon	Locality	County	Sample Type	Collector
KS01	<i>K. s. longifem orale</i>	Presa Xochimilco	Sonora, Mex.	blood	P. Rosen
KS02	<i>K. s. longifem orale</i>	Presa Xochimilco	Sonora, Mex.	blood	P. Rosen
KS03	<i>K. s. longifem orale</i>	Presa Xochimilco	Sonora, Mex.	blood	P. Rosen
KS04	<i>K. s. longifem orale</i>	Presa Xochimilco	Sonora, Mex.	blood	P. Rosen
KS05	<i>K. s. longifem orale</i>	Presa Xochimilco	Sonora, Mex.	blood	P. Rosen
KS06	<i>K. s. longifem orale</i>	Presa Xochimilco	Sonora, Mex.	blood	P. Rosen
KS07	<i>K. s. longifem orale</i>	Presa Xochimilco	Sonora, Mex.	blood	P. Rosen
KS08	<i>K. s. longifem orale</i>	Presa Xochimilco	Sonora, Mex.	blood	P. Rosen
KS09	<i>K. s. longifem orale</i>	Presa Xochimilco	Sonora, Mex.	blood	P. Rosen
KS10	<i>K. s. longifem orale</i>	Presa Xochimilco	Sonora, Mex.	blood	P. Rosen
KS11	<i>K. s. longifem orale</i>	Quitovac	Sonora, Mex.	blood	P. Rosen
KS12	<i>K. s. longifem orale</i>	Quitovac	Sonora, Mex.	blood	P. Rosen
KS13	<i>K. s. longifem orale</i>	Quitovac	Sonora, Mex.	blood	P. Rosen
KS14	<i>K. s. longifem orale</i>	Quitovac	Sonora, Mex.	blood	P. Rosen
KS15	<i>K. s. longifem orale</i>	Quitovac	Sonora, Mex.	blood	P. Rosen
KS16	<i>K. s. longifem orale</i>	Quitovac	Sonora, Mex.	blood	P. Rosen
KS17	<i>K. s. sonoriense</i>	Cienega Creek	Pima	clip	D. Caldwell
KS18	<i>K. s. sonoriense</i>	Aliso Cyn	Santa Cruz	clip	D. Caldwell
KS19	<i>K. s. sonoriense</i>	Cienega Creek	Pima	swab	C. Goldberg
KS20 *	<i>K. s. sonoriense</i>	San Rafael	Santa Cruz	* unsuccessful	P. Rosen
KS21	<i>K. s. longifem orale</i>	Quitoba quito	Pima	blood	D. Riedle
KS22	<i>K. s. longifem orale</i>	Quitoba quito	Pima	blood	D. Riedle
KS23	<i>K. s. longifem orale</i>	Quitoba quito	Pima	blood	D. Riedle
KS24	<i>K. s. longifem orale</i>	Quitoba quito	Pima	blood	D. Riedle
KS25	<i>K. s. longifem orale</i>	Quitoba quito	Pima	blood	D. Riedle
KS26	<i>K. s. longifem orale</i>	Quitoba quito	Pima	blood	D. Riedle
KS27	<i>K. s. sonoriense</i>	Texas Cyn, Chiricahua Mts	Cochise	blood	D. Riedle
KS28	<i>K. s. sonoriense</i>	Texas Cyn, Chiricahua Mts	Cochise	blood	D. Riedle
KS29	<i>K. s. sonoriense</i>	Texas Cyn, Chiricahua Mts	Cochise	blood	D. Riedle
KS30	<i>K. s. sonoriense</i>	Texas Cyn, Chiricahua Mts	Cochise	blood	D. Riedle
KS31	<i>K. s. sonoriense</i>	Skeleton Cyn, Peloncillo Mts	Cochise	blood	D. Riedle
KS32	<i>K. s. sonoriense</i>	Skeleton Cyn, Peloncillo Mts	Cochise	blood	D. Riedle
KS33	<i>K. s. sonoriense</i>	Geronomo Tank, Peloncillo Mts	Hidalgo (N.M.)	blood, toe clip	D. Riedle
KS34	<i>K. s. sonoriense</i>	Hassayampa R., NW of Phoenix	Maricopa	blood, toe clip	D. Riedle
KS35	<i>K. s. sonoriense</i>	Gun Cr. Near Young	Gila	toe	B. Burger
KS36	<i>K. s. sonoriense</i>	Gun Cr. Near Young	Gila	toe	B. Burger
KS37	<i>K. s. sonoriense</i>	Walnut Creek near Young	Gila	toe	B. Burger
KS38	<i>K. s. sonoriense</i>	Sycamore Creek	Maricopa	mummified turtle	D. Riedle
KS39	<i>K. s. sonoriense</i>	Sabino Canyon, near Tucson	Pima	blood	P. Rosen
KS40	<i>K. s. sonoriense</i>	Sabino Canyon, near Tucson	Pima	blood	P. Rosen

Results

Although only 6 base pair substitutions were found within the full sample of *K. sonoriense* from 13 localities (Fig. 2), all samples of *K. s. sonoriense* were grouped together as a derived group with 2 shared substitutions (Fig. 3). A third substitution (position 730) was also shared by all of these turtles and 7 of the 10 sampled in Rio Sonoya mainstem at Presa Xochimilco. The 2 samples from Gun Creek, Gila County, Arizona shared a single unique substitution, and single turtles from the Peloncillo Mountains (New Mexico) and Quitobaquito each had a single unique substitution (Figs. 2, 3). All 12 samples from the isolated springs at Quitovac and Quitobaquito, and 3 of the 10 from Rio Sonoya mainstem shared no substitutions with any of the other *K. sonoriense*. The phylogeny based on parsimony can be simplified to Fig. 4.

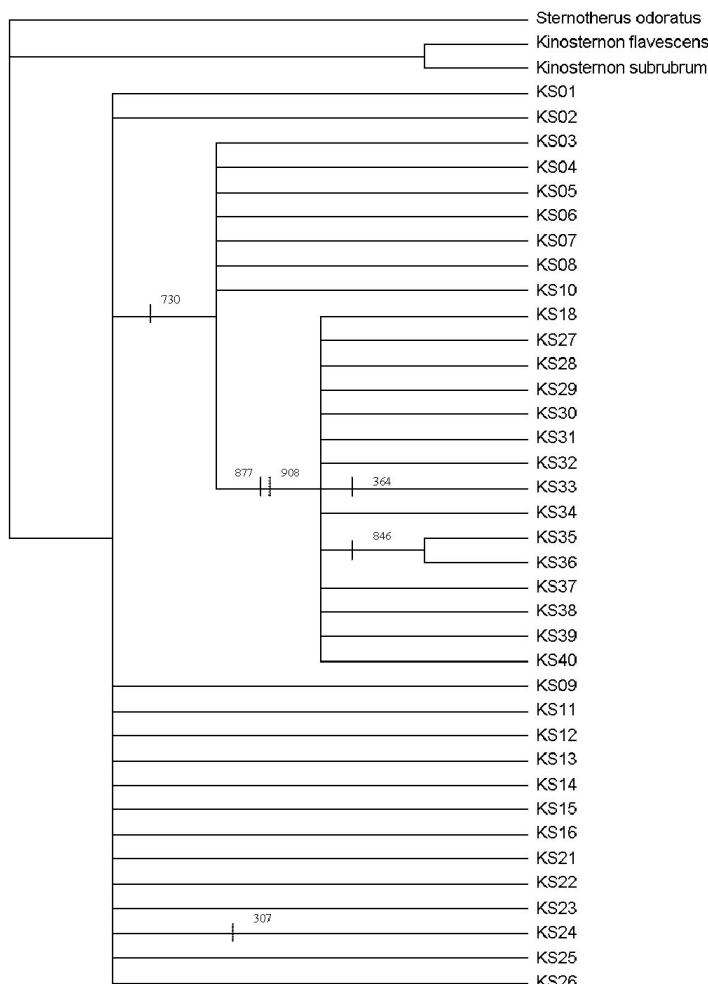


Fig. 2. Sample cladogram for *Kinosternon sonoriense* showing location of base pair substitutions (numbered hash marks) on the mtDNA d-loop. Sample number labels correspond to Table 1.

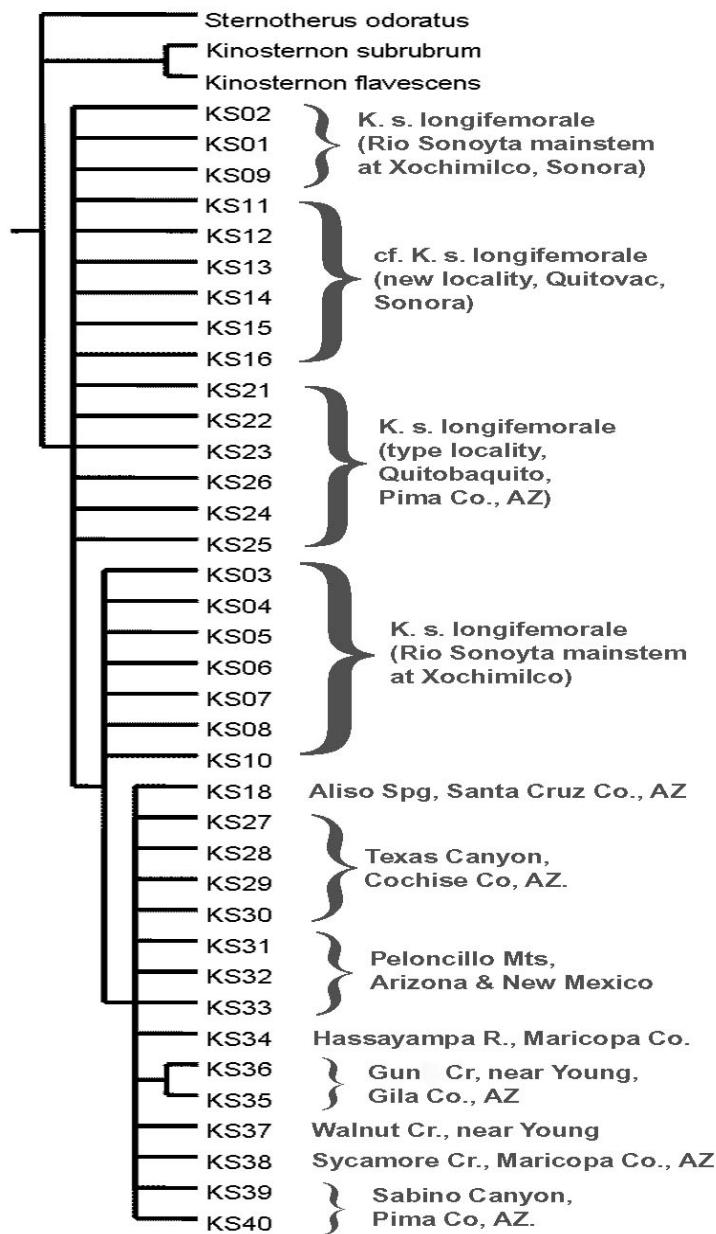


Fig. 3. Sample cladogram for *Kinosternon sonoriense* showing sample localities.

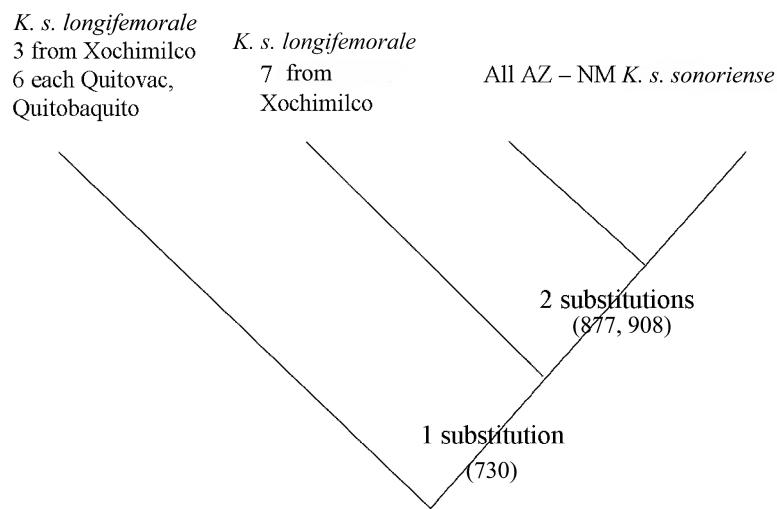


Fig. 4. Simplified cladogram for *Kinosternon sonoriense* from Arizona, New Mexico, and the region of Rio Sonoya.

Discussion

The results of this genetic study clearly indicate that Sonoran Mud Turtles from the Rio Sonoya region (Quitobaquito, Rio Sonoya, and Quitovac), which correspond to Iverson's (1981) Sonoya Mud Turtle (*Kinosternon sonoriense longifemorale*), differ genetically from samples of the nominate subspecies taken from southwestern New Mexico and throughout Arizona. Although only 6 base-pair substitutions were found in the 961 base-pair section of d-loop sequenced, 3 of them are shared by all samples of *K. s. sonoriense* and 2 distinguish all of them from all samples of *K. s. longifemorale*. This supports recognition of the Sonoya Mud Turtle as a distinct taxon.

In modern systematic practice, the smallest fully diagnosable group is frequently accorded full species rank. This would suggest that the Sonoya Mud Turtle could be elevated to full species rank, based on the results presented here. If these results are confirmed, and if present trends in taxonomy continue, that may indeed occur; however, I recommend retaining the current taxonomy for three reasons: (1) the two taxa are clearly very similar and appear to be part of a single biogeographical entity; (2) the biological species concept is still widely accepted outside of currently fashionable systematic practice; and (3) our results suggest a paraphyletic relationship between *K. s. sonoriense* and *K. s. longifemorale*, possibly as a result of reticulation, and it seems preferable to recognize the potentially reticulated evolutionary lineage (i.e., a history that may have involved hybridization of diverging lineages) as a phenomenon that occurred within a species.

Paraphyly is indicated in Fig. 4, wherein 7 individuals from Rio Sonoya mainstem share a synapomorphy (a shared derived character) with *K. s. sonoriense*. Based on this information, they could be included in that subspecies, and would be inferred to share a more recent common

ancestor with *K. s. sonoriense* than with *K. s. longifemorale* (including some individuals from the same population at Presa Xochimilco, which lacked this substitution). If *K. s. longifemorale* was elevated to species rank, the base-pair substitution at position 730 creates a situation in which a character polymorphic within a population allocates some individuals to one species and others to another, which is untenable within morphological and geographic contexts. We could choose to ignore the character (as uninformative) or we could seek other explanations for the observed character state distribution.

One option would be to argue that the mainstem Rio Sonoya population is ancestral to all others currently in *K. sonoriense*, and that the polymorphism is ancestral. This is certainly tempting but all of the following must be true for this to be true: (1) the origination of *K. sonoriense* must have involved deriving the substitution at 730 from the putative ancestor, which lacked it, (2) the polymorphic must have persisted in the mainstem over the long temporal period of the species existence, (3) distinctive populations at both Quitovac and Quitobaquito must have been derived, presumably independently by losses of the polymorphism at position 730, or they must have become isolate prior to the polymorphism arising in the mainstem, and (4) this same substitution must have been fixed, again independently of the converse fixation(s) at Quitovac and Quitobaquito (see Fig. 2). In addition to the substantial number of unsupported logical requirements in this scenario, it is additionally unlikely that such a peripheral isolate as that at Rio Sonoya at the most arid margin of a broadly distributed, evolving taxon would be an ancestor in this real sense, rather than simply retain plesiomorphous character states by virtue of being isolated from the main stream of evolution within the species. In other words, it is more likely to retain character states once widely distributed in the ancestral population than to be the center of origin for the entire species.

There are other, somewhat more plausible explanations for the observed polymorphism at position 730 at Presa Xochimilco. (i) Larger samples might reveal its presence as a polymorphic character in other populations of *K. s. longifemorale*, dulling the edge of Occam's Razor in the argument above. (ii) We could note that small population sizes in the isolated springs at Quitobaquito and Quitovac may have led to loss of one of the variants at this position, also weakening the parsimony argument. Even under these two arguments, we would still have to argue that the polymorphism arose in *K. s. longifemorale* and failed to go to fixation in that subspecies over the long history of the species. (iii) The character would simply be misinformative on the relationships, as a result of homoplasy (parallelism), which is possible but not parsimonious based on the limited available data. (iv) Re-contact between the evolving nominate subspecies and the mainstem population may have occurred, injecting the substitution at 730 into *K. s. longifemorale* at the point of contact, but not necessarily affecting the isolated spring populations.

Any of the explanations (i - iv) are possible, but (iv) seems most parsimonious, and is plausible on biogeographical grounds. The re-contact hypothesis (iv) can explain all the existing data under the single stringent logical requirement that the re-contact occurred at a time when substitution 730 had occurred (or at least arisen) in the *K. s. sonoriense* gene pool, but the substitutions at 877 and 908 had not been completed. Although this hypothesis also, obviously, requires that habitat conditions have varied and gene flow would thus have been permitted, these are not stringent requirements since we know with certainty that less arid conditions prevailed during interglacial periods that were repeated throughout the Pleistocene. Fig. 5 diagrams drainage patterns that could have permitted interchange between *K. s. longifemorale* in Rio Sonoya and turtles from the evolving nominate subspecies in Gila River basin. Although these connecting regions are currently without perennial or semi-perennial turtle habitat, and are occupied by a form of yellow mud turtle (the Arizona Mud Turtle, *K. (flavescens) arizonense*, recently elevated to specific status by Serb et al. [2001]) that lives in ephemeral summer rain

pools, contact between the taxa within *K. sonoriense* does not seem implausible given the semi-perennial conditions under which we have found *K. s. longifemorale*. Under the currently hot, arid climate, the subspecies is closely centered on sites where shallow rock creates perennial springs, but we can infer that with more rainfall and less evaporation, semi-perennial to perennial conditions would have become more widespread away from these rock dykes. The most likely place we would see traces of such contact would be at Sonoyta, precisely where the polymorphism at position 730 was found. Exchange that may have occurred in the other direction would have likely gotten swamped within the larger Gila River basin population, or evidence of it may have disappeared along with the apparent anthropogenic extinction of the species in the lower Gila and Colorado rivers. The re-contact scenario is also consonant with the idea that these two taxa are quite similar, and are likely to intermingle and interbreed freely should they come into geographical contact. However, it must be emphasized that the small number of informative substitution sites makes this discussion speculative, and that more sequences must be obtained, sample sizes should be expanded somewhat, and samples from the nominate subspecies in Sonora must be evaluated before we can reach conclusions about the detailed evolutionary history within *K. sonoriense*.

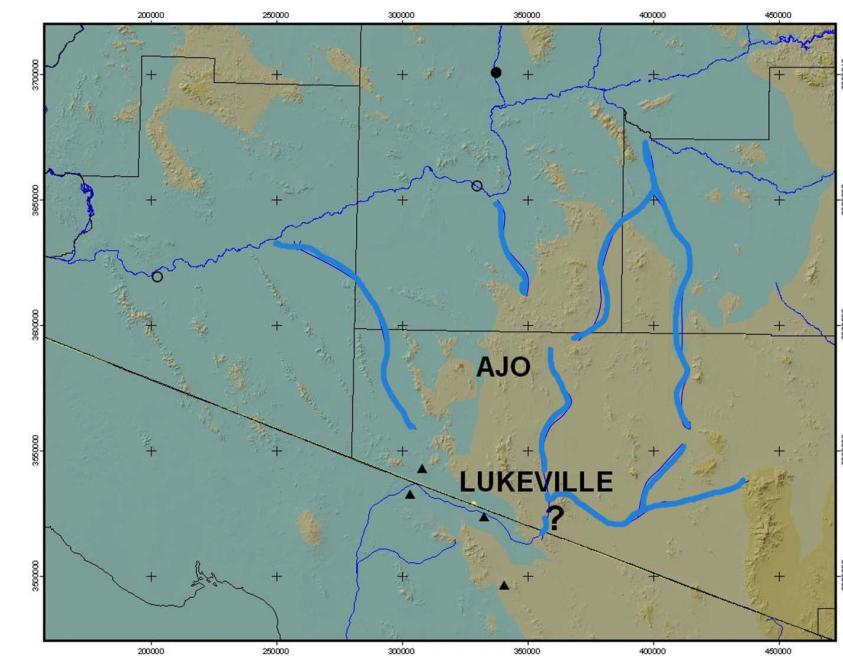


Fig. 5. Possible routes of contact between the Sonoyta Mud Turtle (*Kinosternon sonoriense longifemorale*, triangles) and the nominate subspecies (*K. s. sonoriense*, solid circles, with apparently extirpated populations shown as hollow circles). Large channels of major drainages are shown in light blue, while currently ephemeral drainageways that may have provided avenues for contact during wetter interglacials are marked with heavier blue

Avise et al. (1992) presented evidence that turtle mtDNA evolves more slowly than that of most other vertebrate groups that have been evaluated. They estimated the rate of base-pair substitution at approximately 0.25 % per million years. Assuming the re-contact scenario argued above, the two subspecies have achieved 0.31 % (3/961) sequence divergence, suggesting an ancient initial divergence at around 1.24 million years ago, which is remarkable for two rather similar-looking beasts. Various caveats must be noted here (small sample size, lack of reliability

of the molecular clock), but the data suggest the divergence has some antiquity.

Echelle et al. (2000) estimated that divergence between the Quitobaquito pupfish and the desert pupfish (*C. (m.) macularius*) in Colorado River basin occurred about 20,000 years bp, much more recently than the suggested divergence of the Sonoya Mud Turtle. The Sonoran Mud Turtle is more capable of living under marginally perennial conditions than the pupfish, so the re-contact hypothesis suggested for the turtles need not apply to the fish. However, if Rio Sonoya flowed into the Colorado River delta region prior to recent vulcanism in the Pinacate region, both taxa should have been in contact: pupfish and mud turtles should show relatively more similar patterns of divergence than they do. An alternative is that the pupfish's salinity tolerance may allow limited contact between the Colorado delta and Rio Sonoya along the Sonoran coast, perhaps facilitated during wetter times by increased discharge of fresh water from the rivers, or by more widespread occurrence of sloughs or estuaries inhabitable by pupfish but not mud turtles. An evaluation of genetic divergence of longfin dace in Rio Sonoya could shed light on this, as this species is unlikely to tolerate salinity like the pupfish.

In summary, results of this study support recognition of the Sonoya Mud Turtle (*Kinosternon sonoriense longifemorale* Iverson) as a valid and distinctive taxon that may be threatened by human activities within its limited range. Questions about the validity of this taxon cannot be unambiguously answered until (1) samples from a range of sites within Sonora are included, and (2) ND4 and perhaps one additional gene are sequenced and added to the analysis, to increase sample size and review the generality of what has been learned from the d-loop.

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APPENDIX. Gene sequences from mtDNA d-loop from three outgroups, *Kinosternon sonoriense longifemorale* from Sonora and Arizona and *K. s. sonoriense* from Arizona and southwestern New Mexico.

	0	1	2	3	4	5
0	1	2	3	4	5	
1	2	3	4	5	6	7
2	3	4	5	6	7	8
3	4	5	6	7	8	9
4	5	6	7	8	9	0
5	6	7	8	9	0	1
6	7	8	9	0	1	2
7	8	9	0	1	2	3
8	9	0	1	2	3	4
9	0	1	2	3	4	5
S.od	ATAAAAGATAACGCAT-TTTACACCATA	TTT	TAGCCGCATT	CCCCCTACCTAGTT		
K.fl	ATAAAAGATAACGCATCTTGCA	CCCAT	TTT	TAGCCGTATT	CCCCCTACCTAGTC	
K.su	ATAAAAGATAACGCATCTTACACC	AT	TTT	TAGCCGTATT	CCCCCTACCTAGTC	
KS01	ATAAAAGATAACGCATCTTACGCC	AT	TTT	TAGCCGTATT	CCCCCTACCTAGTC	
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5	6	7	8	9	10
5	6	7	8	9	10
5	6	7	8	9	10

3
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9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8

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4				5																	
7	8		9		0		1		2												
4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5

5
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6
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